# Wet Fractionation of Alfalfa Juice for Value-Added Products

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### Introduction

Biotechnologists at the University of Wisconsin have created transgenic alfalfa varieties which produce industrially valuable enzymes not normally produced in alfalfa. These include alpha-amylase, widely used in converting starch to sugar, and manganese-dependent peroxidase, believed to be valuable for the bio-pulping of wood in the paper industry. Much "environmentally-friendly" processing envisaged for the future depends on an abundant and inexpensive supply of enzymes. An example of this is the hydrolysis of biomass to sugars which are fermentable to ethanol, usable as a transportation fuel.

The enzymes phytase and cellulase are currently being added to alfalfa. The former allows phosphorus in the rations of monogastrics to be used more efficiently which also results in less phosphorus loading of the environment. The latter is important for the hydrolysis of ligno-cellulosics to fermentable sugars in the production of ethanol.

Target enzymes will be harvested from the juice of transgenic alfalfa. This juice contains two forms of protein: particulate (or chloroplastic) and soluble (or cytoplasmic). Since the target enzymes generally occur with the soluble protein fraction, maximizing the yield of this fraction is of interest. In addition, the soluble fraction has potential as a protein fortifier in food products. The chloroplastic fraction contains pigmenting agents, called xanthophylls, which are valued in the poultry industry to color yolks and skin. The xanthophylls in the particulate protein concentrate give it a potential value of 2½ to 3 times that of its protein value alone based on the cost of currently used pigmenting materials. The fibrous fraction, in addition to being valuable as a ruminant feed, also has potential as a biofuel either via direct combustion (~8000 BTU/ lb DM) or by conversion to ethanol.

For estimating the economic potential of the wet fractionation process, it is necessary to know approximate yields of the various products.

### **Methods**

Four 15'x 30' plots were laid out in an alfalfa field which had been seeded the previous year. The four plots were harvested May 22, May 29, June 8, and June 16, respectively. Each plot was subsequently cut at intervals of 30-36 days with the exception of the fourth cuttings which were delayed to allow the plants to build root reserves.

Herbage from each cutting was prepared in a rotary impact macerator and juice was expressed in a 6" diameter Rietz Screw press.

#### Results

Dry matter yields for both the herbage and the juice are given in Table 1. The average seasonal dry matter per acre yields for herbage and juice were 6.02 tons and 1.89 tons, respectively, for an overall juice/herbage yield of 0.31.

The fiber fraction of plot 3, first cutting, had the following analysis: protein 9.9%; NDF, 67.7% and ADF, 60.0%, indicating high level of solubles extracted in the juice.

If the juice-derived particulate protein concentrate (~ 45% protein) and the soluble protein concentrate (~ 90% protein) yields are approximately .41 and .15, respectively (Koegel and Straub 1994) of the juice dry matter, the seasonal per acre yields of the concentrates would be .077 tons and .28 tons, respectively, and the protein yields would be 0.35 tons and 0.26 tons, respectively, for a total protein yield of 0.61 tons. If yield targets for the target enzymes of 1 - 5% of the soluble protein were attained, then 5.2 - 26.0 lb per acre of enzymes could be "harvested".

The per acre energy content of the fibrous fraction would be about 66 million BTU. If converted to electricity at an efficiency of .33, this would result in 6385 KWh or power of 100KW for over 2½ days.

## **Conclusions**

Since the yield results shown are for a single set of plots for one year, these would be considered highly tentative. Staging the cutting of plots over a period of 4½-5 weeks to allow better utilization of equipment in a field-scale operation appeared promising in 1995.

Since relatively aggressive processing was used for juice expression, the juice dry matter yield of 0.3 herbage dry matter is probably close to the maximum attainable (unless rewetting and repressing is used).

# Reference

Koegel, R.G. and R.J. Straub. 1994. Fractionation of alfalfa for food, feed, biomass, and enzymes. ASAE Paper #946010.

Table 1: Dry matter yields for alfalfa herbage and juice - 1995. ( ) = cutting date.

|      | Cutting 1           |                        | Cutting 2           |                        | Cutting 3              |                        | Cutting 4           |                        | Season              |                   |            |
|------|---------------------|------------------------|---------------------|------------------------|------------------------|------------------------|---------------------|------------------------|---------------------|-------------------|------------|
| Plot | Herbage DM (t/acre) | Juice DM<br>Herbage DM | Herbage DM (t/acre) | Juice DM<br>Herbage DM | Herbage DM<br>(t⁄acre) | Juice DM<br>Herbage DM | Herbage DM (t/acre) | Juice DM<br>Herbage DM | Herbage DM (t/acre) | Juice DM (t/acre) | Herbage DM |
| 1    | 1.42<br>(5/22)      | .34                    | 1.31<br>(6/22)      | .32                    | 1.62<br>(7/28)         | .34                    | 1.17<br>(9/1)       | .34                    | 5.52                | 1.83              | .33        |
| 2    | 1.99<br>(5/30)      | .30                    | 1.41<br>(6/29)      | .30                    | 1.38<br>(8/3)          | .33                    | 1.03<br>(9/11)      | .37                    | 5.81                | 1.82              | .31        |
| 3    | 2.45<br>(6/8)       | .31                    | 1.34<br>(7/10)      | .32                    | 1.29<br>(8/14)         | .31                    | 0.79<br>(10/18)     | .38                    | 5.87                | 1.88              | .32        |
| 4    | 3.26<br>(6/16)      | .25                    | 1.80<br>(7/20)      | .30                    | 1.18<br>(8/24)         | .31                    | 0.65<br>(10/18)     | .47                    | <u>6.89</u>         | 2.02              | <u>.29</u> |
|      |                     |                        |                     |                        |                        |                        |                     | Avg.                   | 6.02                | 1.89              | .31        |